Preclinical validation of the micropipette-guided drug administration (MDA) method in the maternal immune activation model of neurodevelopmental disorders

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ABSTRACT

Pharmacological treatments in laboratory rodents remain a cornerstone of preclinical psychopharmacological research and drug development. There are numerous ways in which acute or chronic pharmacological treatments can be implemented, with each method having certain advantages and drawbacks. Here, we describe and validate a novel treatment method in mice, which we refer to as the micropipette-guided drug administration (MDA) procedure. This administration method is based on a sweetened condensed milk solution as a vehicle for pharmacological substances, which motivates the animals to consume vehicle and/or drug solutions voluntarily in the presence of the experimenter. In a proof-of-concept study, we show that the pharmacokinetic profiles of the atypical antipsychotic drug, risperidone, were similar whether administered via the MDA procedure or via the conventional oral gavage method. Unlike the latter, however, MDA did not induce the stress hormone, corticosterone. Furthermore, we assessed the suitability and validity of the MDA method in a mouse model of maternal immune activation, which is frequently used as a model of immune-mediated neurodevelopmental disorders. Using this model, we found that chronic treatment (> 4 weeks, once per day) with risperidone via MDA led to a dose-dependent mitigation of MIA-induced social interaction deficits and amphetamine hypersensitivity. Taken together, the MDA procedure described herein represents a novel pharmacological administration method for *per os* treatments in mice that is easy to implement, cost effective, non-invasive, and less stressful for the animals than conventional oral gavage methods.

1. Introduction

In-vivo animal models remain a cornerstone of preclinical research. One main goal of modeling a disease is to achieve a more profound understanding of its etiology and pathophysiology, which in turn may identify possible targets for its treatment and/or optimize existing treatment options. Pharmacological treatments in animal models are also critical for ascertaining the predictive validity of a model, that is, the extent to which pharmacological compounds that are known to influence a clinical state in humans have a similar effect in the animal model (Peleg-Raibstein et al., 2012).

Depending on the characteristics of the treatment (solubility of the compound, pharmacokinetics, pharmacodynamics, and length of treatment), experimenters can implement a variety of different administration routes, all of which have their own advantages and drawbacks. The latter can be substantial when considering chronic administration regimes. For example, repeated restraint of the experimental animal represents a chronic stressor to the animal, which in turn can confound the results of the study (Vandenberg et al., 2014). Besides inducing stress, chronic intraperitoneal (i.p.) or subcutaneous (s.c.) injections can also lead to infections at the sites of injections, whereas repeated oral gavages (o.g.) can cause perforation of the esophagus, trachea or stomach (Arantes-Rodrigues et al., 2012; Burkholder et al., 2012; Turner et al., 2011; Vandenberg et al., 2014). Thus, these administration routes are suboptimal for chronic treatment regimens, especially in small laboratory animals such as the mouse (Burkholder et al., 2012; Turner et al., 2011).

By contrast, providing substances in food or drinking water is a non-

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stressful and non-invasive method that is commonly used for chronic *per os* administration of substances (Turner et al., 2011). Because the animals have constant access to the substance, however, the plasma levels of the substance remain relatively constant throughout their active phase but decline during the non-active phase (Delotterie et al., 2009; Gao et al., 2009, 2005; Löscher and Schmidt, 1988; Meyer et al., 2010). Therefore, one limitation of *per os* administration of substances via food or drinking water is that it can lead to a plasmatic profile that is different to that obtained in human oral administrations, where substances are typically administered at discrete administration windows and, depending on the substance, lead to a concentration peak in the blood at specific time intervals (Kapur et al., 2003; Shaywitz et al., 1982). Moreover, administration of a substance in the drinking water or food requires constant titration of its concentration in order to accommodate how much the animal weighs, eats, and drinks. Lastly, when considering administration in the drinking water, this method requires the drug to be soluble and stable in this vehicle, restricting this method to only certain compounds.

The use of minipumps, which can be inserted into the peritoneal cavity or subcutaneously, is a minimally invasive alternative to providing substances in drinking water or food for chronic treatments (Gensler et al., 2012). Minipumps can deliver a preloaded solution continuously at rates between 1 and 34 μL/min (Gensler et al., 2012), thereby reducing the amount of daily stress for the animals. This method guarantees constant levels of drug exposure, but it requires the animal to undergo initial surgery for the minipump insertion and, in many cases, necessitates post-surgical analgesic treatments (Gensler et al., 2012; Theeuwes and Yum, 1976). Moreover, minipumps pose a problem for their size and for poorly soluble compounds, and, similar to *per os* administration of substances via food or drinking water, they can lead to plasmatic profiles that are different to those obtained by oral administrations at specific time intervals (Nau, 1986). Following a similar principle to that of minipumps, slow-release pellets implanted subcutaneously allow for chronic treatment with minimal experimenter influence. While the animal is under general anesthesia, the pellets are inserted subcutaneously in the scruff of the neck, where they slowly dissolve, allowing the drug to enter the bloodstream over the course of a set time frame with zero order kinetics (Bloomfield et al., 2018). The duration of release varies from one to several weeks, depending on the dimensions of the pellet and on the released compound. As a consequence, in chronic studies longer than 21 days, pellets may have to be replaced weekly, causing both stress to the animals and risks of infection at the insertion site with consequent dropout of experimental subjects (Gasparini et al., 2016). Moreover, while some compounds delivered through slow-releasing pellets reach a human-like steady-state concentration (Bloomfield et al., 2018; Crowley et al., 2012; Kim et al., 2018), others show a pattern of rapid high plasmatic concentration during the first days after implantation, which then steadily decreases over the following days (Gasparini et al., 2016; McLane et al., 2017). Lastly, both osmotic minipumps and slow-release pellets are characterized by relatively high costs that render them less cost-effective as compared, for example, to administration via the drinking water (Gasparini et al., 2016).

Taken together, all of the above-mentioned methods have inherent limitations when adopted for chronic pharmacological treatments in laboratory animals. Therefore, the present study was designed with the aim to develop and validate a novel administration method that minimizes these limitations while being applicable for chronic *per os* treatments in mice. This method, which we hereby refer to as micropipette-guided drug administration (MDA) procedure, is based on the use of a palatable solution in the form of sweetened condensed milk mixed with water. In this procedure, the animals are trained to ingest the palatable drug (or vehicle) solution in a controlled manner in the presence of the experimenter. The MDA technique allows repeated administration of substances without the need for a full restraint or invasive techniques, thus drastically minimizing the stressful impact on the experimental animals and on the experimenter.

In order to validate the novel MDA method, we first compared the pharmacokinetic profiles of the commonly used anti-psychotic drug, risperidone (RIS), when administered via traditional oral gavage and via MDA in mice. Risperidone is not readily soluble in water, rendering it an ideal candidate for validating the suitability and efficiency of the MDA method. Subsequently, we assessed the suitability and validity of the MDA method in a mouse model of maternal immune activation (MIA), which is frequently used as a model of immune-mediated neurodevelopmental disorders in numerous species, including mice (Brown and Derkits, 2010; Brown and Meyer, 2018; Estes and McAllister, 2016). This MIA model incorporates an established risk factor of various neurodevelopmental disorders (Brown and Derkits, 2010; Brown and Meyer, 2018) and is based on maternal administration of the viral mimetic poly(I:C) (=*polyriboinosinic-polyribocytidilic acid*). Prenatal poly(I:C) exposure in laboratory mice and other species leads to multiple changes in brain functions and behavior with relevance to neurodevelopmental and psychiatric disorders, such as autism spectrum disorder and schizophrenia (Careaga et al., 2017; Harvey and Boksa, 2012a, 2012b; Meyer, 2014; Meyer et al., 2009). Adopting the MDA method in the MIA model, we examined whether chronic RIS treatment in adulthood could mitigate several poly(I:C)-induced behavioral deficits, including impairments in social approach behavior, attenuation of sensorimotor gating, and hyper-responsiveness to acute dopaminergic stimulation (Meyer, 2014; Meyer et al., 2009).

2. Materials and methods

2.1. Animals

C57BL6/N mice (Charles River Laboratories, Sulzfeld, Germany) were used throughout the study. They were caged 3–5 animals per cage and sex in individually ventilated cages (IVCs) and kept under a reversed light–dark cycle (lights off: 09:00 AM–09.00 PM) as described before (Mueller et al., 2018) and in the Supplemental Information. All procedures described in the present study had been previously approved by the Cantonal Veterinarian's Office of Zurich, and all efforts were made to minimize the number of animals used and their suffering.

2.2. Micropipette-guided drug administration (MDA) procedure

The MDA procedure was based on the use of a palatable solution in the form of sweetened condensed milk (MIGROS Kondensmilch™, Migros, Zurich, Switzerland) mixed with regular tap water. The condensed milk contained unskimmed cow's milk (59%), sugar (55 g per 100 g), and stabilizer E339. It was diluted with water, yielding to a 3:10 (condensed milk to water) ratio. The water-diluted condensed milk solution was used as vehicle for risperidone (RIS) throughout the study. RIS was first suspended in distilled water and 0.5% hydroxyethylcellulose, placed in an ultrasonic water bath, and sonicated for 20 min before being mixed with the vehicle (i.e., the water-diluted condensed milk solution). The doses of RIS ranged between 0.4 and 0.8 mg/kg in the pharmacokinetic studies and included doses of 0.05 and 0.1 mg/kg in the MIA model (see below). The drug and vehicle solutions were kept in constant agitation using magnetic stirrer (Stuart UC152, Carl Roth, Karlsruhe, Germany) prior to administration via MDA.

Vehicle and drug solutions were administered using a volume of 2 ml/kg and were provided with a conventional single channel p200 micropipette (Gilson Pipetteman, Thermo Fischer Scientific, Reinach, Switzerland). Prior to the start of the actual drug or vehicle treatment, all animals were first trained to obtain the condensed milk solution from the micropipette on two consecutive days, with one training session per day. On the first day of training, the mice were fully restrained and exposed to the milk solution for the first time, as the pipette tip was offered to the mouth until the mouse began to drink (Fig. 1A,

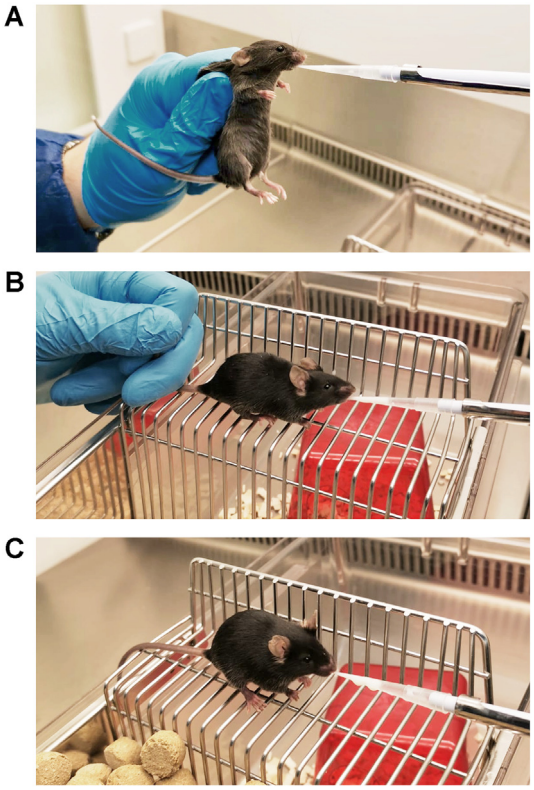

Fig. 1. Phases of the micropipette-guided drug administration (MDA) method in C57BL6/N mice. (A) First day of MDA training: Mice are fully restrained and exposed to the sweetened condensed milk solution for the first time via a conventional micropipette (see also Supplementary Video 1). (B) Second day of MDA training: Mice are restrained solely by the tail and exposed to the sweetened condensed milk solution via a micropipette (see also Supplementary Video 2). (C) Third day of MDA training (when the actual treatment would start): Mice are no longer restrained and drink the sweetened condensed milk solution voluntarily from the micropipette (see also Supplementary Video 3).

Supplementary Video 1). On the second day of training, a looser restraint was used, such that the mice were restrained solely by the tail on the metal grid of the food hopper (Fig. 1B, Supplementary Video 2). Again, the pipette was continually positioned next to the mice's mouth until they drank. On the third day, by the time the treatment began (i.e., two days after the first training session), the mice no longer required any restraint for them to voluntarily drink the solution (Fig. 1C, Supplementary Video 3). For the assessment of the acute stress responses, weight gain, and therapeutic effects of RIS in the MIA model (see below), two different experimenters were assigned to conduct the oral gavage and/or MDA procedures in order to counterbalance potential experimenter bias. Hence, half the animals in each treatment group was treated by one experimenter, and the other half was treated by a different experimenter.

2.3. Pharmacokinetic studies

A proof-of-concept study was conducted to assess the pharmacokinetics of RIS administration using the MDA procedure. To this aim, RIS was administered either using MDA as described above, or via the conventional oral gavage route. When administering RIS via oral gavage, the drug was suspended in distilled water and 0.5% hydroxyethylcellulose, placed in an ultrasonic water bath, sonicated for 20 min, and kept under agitation until treatment (the time between sonication and treatment did not exceed 10 min). Two doses of RIS were chosen (0.4 and 0.8 mg/kg) based on previous uses of RIS in mouse models (Kohlhaas et al., 2012), and administered as described in the Supplementary Information. Blood samples were taken via tail vein sampling after 30, 60, and 90 min after RIS administration, and centrifuged at 4 °C for 10 min to separate the plasma, which was stored at −70 °C. Plasma concentrations of RIS were determined using standard tandem liquid chromatography mass spectrometry (LC-MS/MS). For the pharmacokinetic studies, adult (10–12-week-old) male C57BL6/N mice (Charles River Laboratories, Sulzfeld, Germany) were used. They were kept as described above.

2.4. Assessment of acute stress response

A first experiment was designed to assess whether the MDA procedure might differ from the conventional oral gavage method in terms of inducing stress. To this end, we measured plasma corticosterone (CORT) levels 30 min after MDA or oral gavage in adult (10–12-week-old) male C57BL6/N mice (Charles River Laboratories, Sulzfeld, Germany). Two groups of animals were habituated to either treatment method for 6 days, and one group of animals was kept undisturbed and used as baseline. CORT was then measured after treatment on the 7th day as described before (Mueller et al., 2018).

In a second experiment, we measured CORT levels during the learning phase of the MDA procedure, that is, 30 min after MDA administration on day 1 (which requires a full restraint; see Supplementary Video 1), day 2 (which only requires a tail restraint on the food hopper; see Supplementary Video 2) and day 3 (when the animals freely drink from the micropipette without any restraint being used; see Supplementary Video 3). These three groups were compared to animals that were left undisturbed (baseline measures; taken on each day) and a group of animals subjected to oral gavage for the first time (corresponding to day 1 of the MDA procedure). In addition, we measured the time it took the animals to drink the required volume of solution on all of the three days.

For all CORT measures, trunk blood was collected after decapitation. After collection, the blood samples were spun at 4 °C for 10 min to separate the plasma, and plasma CORT was analyzed with the DetectX® Corticosterone Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, USA) according to the manufacturer's instructions.

2.5. Assessment of weight gain during chronic administration with MDA

We also compared the weight gain of male and female C57BL6/N mice (Charles River Laboratories, Sulzfeld, Germany) chronically exposed to the MDA procedure, relative to mice that were left undisturbed, starting from postnatal day (PND) 21 until they reached PND 90. These measurements served to assess whether chronic daily consumption of a limited amount of condensed milk, which is an inherent part of the MDA procedure, might affect the growth and weight of the treated animals.

2.6. Maternal immune activation model

Female and male C57BL6/N breeder mice were exposed to a timed-mating procedure as described previously (Meyer et al., 2005; Mueller et al., 2018) and in the Supplementary Information.

Pregnant dams were subjected to either a single injection of poly (I:C) at a dose of 2.5 mg/kg (potassium salt; Sigma–Aldrich, Buchs, St Gallen, Switzerland, lot #117M4005V) or vehicle on gestation day 12 as described in detail in the Supplementary Information (Mueller et al., 2019, 2018). A total of 28 litters (13 poly(I:C) and 15 vehicle control litters) were prepared in order to minimize possible litter effects (Supplementary Information). The housing and experimental allocation of the poly(I:C)-exposed or control offspring are described in the Supplementary Information. A reporting guideline checklist for the MIA model (Kentner et al., 2019) is also provided in the Supplementary Table S1.

RIS or corresponding vehicle treatment via MDA started when poly

(I:C)-exposed or control offspring reached postnatal day (PND) 70 (Supplementary Fig. S1). Based on initial RIS dose response studies (Supplementary Fig. S2) and previous studies conducted in the MIA and neonatal ventral hippocampal lesion (NVHL) models (Piontkewitz et al., 2011, 2012; Richtand et al., 2006), RIS was given at a dose of 0.05 or 0.1 mg/kg/day in order to minimize possible confounds arising from sedation, and to assess whether the preventive effects of RIS administered at these doses (Piontkewitz et al., 2012, 2011; Richtand et al., 2006) may be extended to therapeutic effects when given chronically in adulthood. Chronic administration of RIS or vehicle via MDA followed procedures as described above and lasted for a total of 6 weeks. Behavioral testing (see below) was conducted during the last three weeks of the treatment, with animals receiving either RIS or vehicle 30 min before testing. Behavioral testing involved the assessment of (1) social approach behavior in a modified Y-maze, (2) sensorimotor gating in the form of prepulse inhibition (PPI) of the acoustic startle reflex, and (3) amphetamine-induced hyperlocomotion in the open field. These tests were selected because they capture core behavioral deficits induced by poly(I:C)-based MIA in mice (Luan et al., 2018; Notter et al., 2018; Weber-Stadlbauer et al., 2017). The testing apparatuses and procedures are described in the Supplementary Information. Both male and female animals were included to examine possible sex-dependent effects.

2.7. Statistical analysis

All data were analyzed using parametric analysis of variance (ANOVA) as described in the Supplementary Information. Whenever appropriate, ANOVAs were followed by Tukey's post-hoc test to control for multiple comparisons. All statistical analyses were performed using SPSS Statistics (version 22.0, IBM, Armonk, NY, USA) and Prism (version 7.0; GraphPad Software, La Jolla, CA, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effects of the treatment method on the stress response, weight gain, and pharmacokinetic profile of risperidone

The animals generally took < 5 s to consume the condensed milk solution from the micropipette in the MDA procure (Fig. 2A). The consumption time was relatively constant (ranging between 1 and 5 s) across days when the animals were considered as a group. When considering individual animals, there was a non-significant day-to-day variation in terms of the time required to consume the condensed milk solution from the micropipette (Fig. 2A).

To examine whether the MDA procedure differs from the traditional method of oral gavage in terms of inducing stress, we measured plasma CORT levels 30 min after MDA or oral gavage. An additional non-treated group of mice was included as a negative control group. As shown in Fig. 2B, the plasma CORT levels in MDA-treated and non-treated mice did not significantly differ on day 1 of the MDA procure, whereas mice subjected to oral gavage for the first time showed significantly higher CORT levels compared to MDA-treated ($p < 0.01$) and non-treated ($p < 0.001$) mice (main group effect ANIOVA: $F_{(2,17)} = 11.99$, $p < 0.001$). To explore whether these differences persist under chronic treatment conditions, we assessed the effects of habituation to the different treatment regimens. As shown in Fig. 2C, the treatment method significantly ($F_{(2,24)} = 11.97$, $p < 0.001$) influenced plasma CORT levels even after 6 days of habituation to either oral gavage or MDA, with mice treated via oral gavage for 6 days showing a two-fold increase in plasma CORT levels compared to mice undergoing the MDA procedure for 6 days ($p < 0.01$), or compared to non-treated mice ($p < 0.001$). Importantly, plasma CORT levels did not differ between non-treated mice and mice receiving the condensed milk solution via MDA for 6 days (Fig. 2C). We also assessed plasma CORT levels during the learning phase of the MDA procedure, and observed that plasma CORT levels did not differ between MDA-treated and non-treated mice during this time. Indeed, CORT levels in MDA-treated mice did not significantly differ from non-treated mice on days 2 and 3 of MDA training (Supplementary Fig. S3).

Compared with non-treated animals, chronic daily intake of the condensed milk solution did not influence body weight gain from PND 21 to 90 in mice assigned to the MDA procedure (Fig. 2D). Using RIS as a proof-of-concept drug, there was also no evidence for the possibility that the pharmacokinetic profile differs between MDA and oral gavage procedures. Indeed, at either dose of RIS (0.4 or 0.8 mg/kg), the plasma exposure (nM) of RIS did not differ between the MDA and oral gavage methods (Supplementary Fig. S4). For both doses and treatment methods examined, plasma exposure of RIS was maximal at the 30-minutes post-treatment sampling interval and subsided afterwards (Supplementary Fig. S4), demonstrating similar pharmacokinetic profiles after MDA and oral gavage.

3.2. Effects of chronic risperidone given via MDA on social deficits in the MIA model

We used the poly(I:C)-based MIA model in mice to validate the utility and effectiveness of the MDA procedure in preclinical animal models of neurodevelopmental disorders. To this aim, we treated adult offspring of poly(I:C)-treated or control mothers chronically (6 weeks) with RIS (0.05 or 0.1 mg/kg/day) or corresponding vehicle via MDA and subjected them to behavioral testing.

First, we assessed the effects of the pharmacological treatment on social approach behavior using a modified version of the three-chamber social interaction test (Notter et al., 2018; Weber-Stadlbauer et al., 2017). Social approach behavior was significantly influenced by both prenatal treatment and drug treatment, as indicated by the significant two-way interaction in the analysis of the social preference index ($F_{(2,81)} = 11.18$, $p < 0.01$). Vehicle-treated MIA offspring showed a significantly ($p < 0.01$) reduced social preference index compared to vehicle-treated control offspring (Fig. 3A). The MIA-induced social deficit was also evident in the analysis of the absolute exploration time, which yielded a significant three-way interaction between prenatal treatment, drug treatment, and object ($F_{(2,81)} = 5.87$, $p < 0.01$). Indeed, whereas vehicle-treated control offspring showed a clear preference for the unfamiliar live mouse relative to the inanimate dummy object ($p < 0.01$), vehicle-treated MIA offspring did not (Fig. 3B).

The analysis of the social preference index further showed that the MIA-induced social deficit was normalized by chronic treatment with the lower (0.05 mg/kg/day) dose of RIS (Fig. 3A). This effect stemmed from a significant reduction ($p < 0.01$) in the relative time MIA offspring spent with the dummy object after chronic treatment with RIS at a dose of 0.05 mg/kg/day (Fig. 3B). By contrast, chronic treatment with the higher (0.1 mg/kg/day) dose of RIS failed to normalize the MIA-induced social deficit but, instead, significantly ($p < 0.01$) reduced the social preference index in control offspring (Fig. 3A). The latter effect was accounted for by the drug's effect on the exploration time with the unfamiliar live mouse, which markedly decreased after chronic treatment with RIS at a dose of 0.1 mg/kg/day, particularly in offspring of control mothers ($p < 0.01$, Fig. 3B).

The between-subject factor of sex did not significantly interact with prenatal treatment and/or drug treatment, neither in the analysis of the social preference index (prenatal treatment × sex: $F_{(1,81)} = 0.01$, $p = 0.97$; prenatal treatment × drug treatment × sex: $F_{(2,81)} = 0.66$, $p = 0.52$), nor in the analysis of the absolute exploration time (prenatal treatment × sex × object: $F_{(1,81)} = 0.01$, $p = 0.98$; prenatal treatment × drug treatment × sex × object: $F_{(2,81)} = 0.45$, $p = 0.65$). Hence, the MIA-induced social deficit and its normalization by the lower dose of RIS were independent of the offspring's sex.

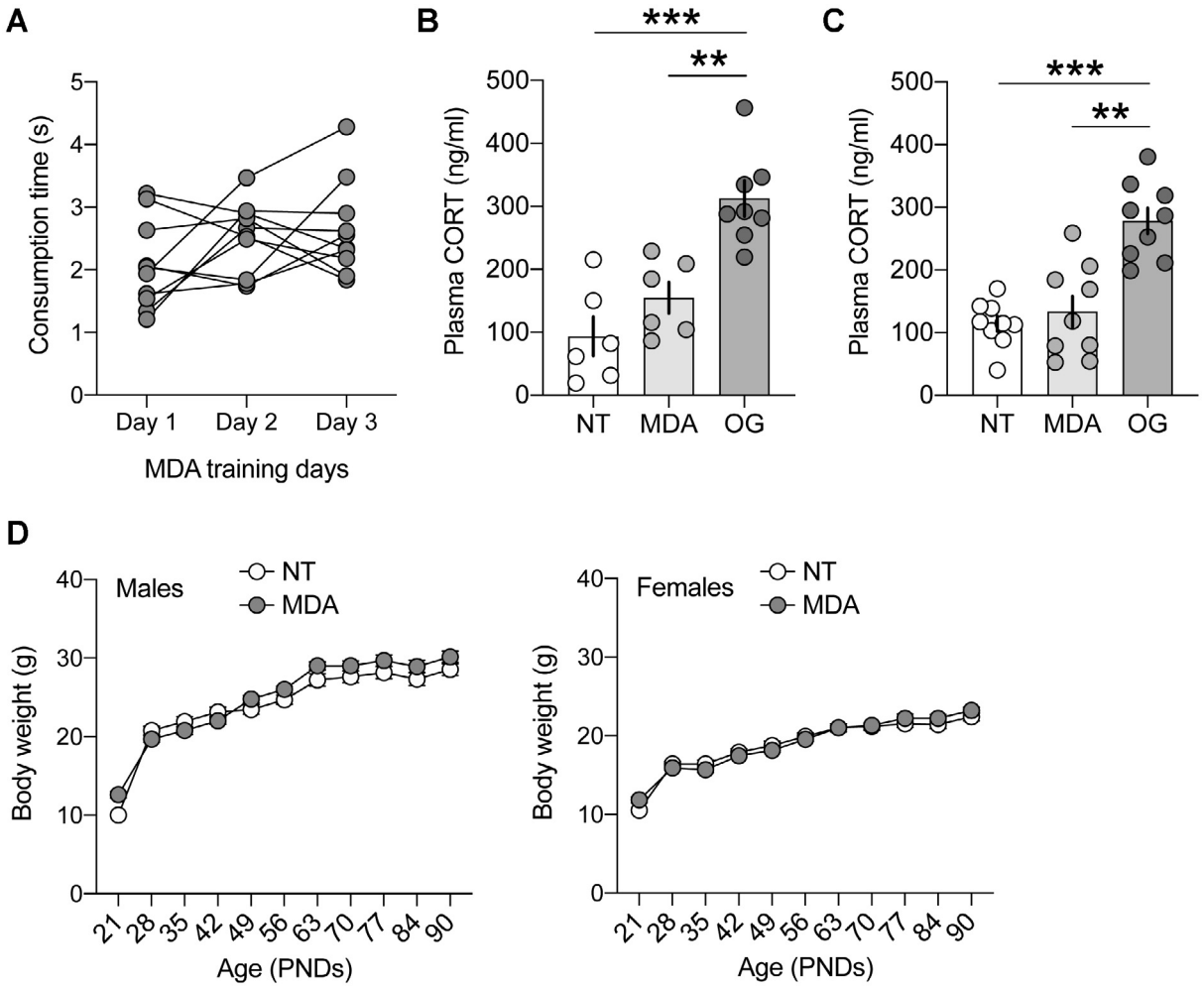

Fig. 2. Effects of the micropipette-guided drug administration (MDA) method on measures of growth and stress. (A) Time needed to consume the sweetened condensed milk solution in the MDA method. The graph shows the performance of individual animals during the three initial days of MDA training. N = 10 male C57BL6/N mice (10–12 weeks of age). (B) Plasma CORT levels 30 min after MDA in male C57BL6/N mice on the first day of MDA training. Non-treated (NT) mice were used as negative control groups, whereas mice treated with conventional oral gavage (OG) were used as a positive control group. $^{**}p < 0.01$ and $^{***}p < 0.001$, based on post-hoc tests following ANOVA. N = 6–8 mice per group. (C) Plasma corticosterone (CORT) levels 30 min after MDA or conventional OG in male C57BL6/N mice after habituation to either treatment method for 6 days. A non-treated (NT) control group of mice that was left undisturbed was used for baseline CORT measures. $^{**}p < 0.01$ and $^{***}p < 0.001$, based on post-hoc tests following ANOVA. N = 9 mice per group. Dots represent individual animals, and error bars represent S.E.M. (D) Body weights in male and female mice (C57BL6/N) assigned to daily MDA treatment starting from postnatal day (PND) 21 to PND 90, relative to mice that were left undisturbed and non-treated (NT) during this period. N = 13 per sex and treatment condition.

3.3. Effects of chronic risperidone given via MDA on sensorimotor gating deficits in the MIA model

Next, we examined the effects of chronic RIS treatment given via MDA on sensorimotor gating deficits in the MIA mouse model (Meyer, 2014; Mueller et al., 2018; Richetto et al., 2013). Sensorimotor gating was measured using the test of prepulse inhibition (PPI) of the acoustic startle reflex (Peleg-Raibstein et al., 2012). In all groups, the % PPI scores increased with increasing prepulse intensities, as supported by the significant main effect of prepulse level ($F_{(2,162)} = 1039.38$, $p < 0.001$) and its interaction with pulse level ($F_{(4,324)} = 11.46$, $p < 0.001$). MIA led to a significant overall reduction in % PPI, as supported by the significant main effect of prenatal treatment ($F_{(1,81)} = 4.71$, $p < 0.05$; Fig. 4A). RIS (0.05 or 0.1 mg/kg/day) did not improve PPI in MIA-exposed offspring, nor did it significantly affect PPI in control offspring (Fig. 4A). Furthermore, there were no significant interactions involving the between-subject factor of sex in the analysis of % PPI (prenatal treatment × sex: $F_{(1,81)} = 0.28$, $p = 0.60$; prenatal treatment × drug treatment × sex: $F_{(2,81)} = 0.02$, $p = 0.98$), indicating that the MIA-induced PPI deficit and lack of normalization by RIS were independent of the sex of the offspring.

There were also no significant main effects or interactions involving the between-subject factors of prenatal treatment and/or drug treatment in the analysis of reactivity to pulse-alone or prepulse-alone trials. In all groups and in both sexes, the reactivity to pulse-alone and prepulse-alone trials increased with increasing pulse (Fig. 4B) and prepulse (Fig. 4C) intensities, respectively (main effect of pulse: $F_{(2,162)} = 184.94$, $p < 0.001$; main effect of prepulse: $F_{(2,162)} = 17.04$, $p < 0.001$).

3.4. Effects of chronic risperidone given via MDA on amphetamine hypersensitivity in the MIA model

We also examined whether chronic RIS treatment given via MDA could normalize the hypersensitivity to acute dopaminergic stimulation by systemic AMPH, which has previously been found in poly(I:C)-based MIA models (Luan et al., 2018; Meyer et al., 2008; Vuillermot et al., 2010; Zuckerman et al., 2003). Neither MIA nor chronic treatment with RIS given at 0.05 or 0.1 mg/kg/day affected basal locomotor activity during the initial habituation or subsequent vehicle administration

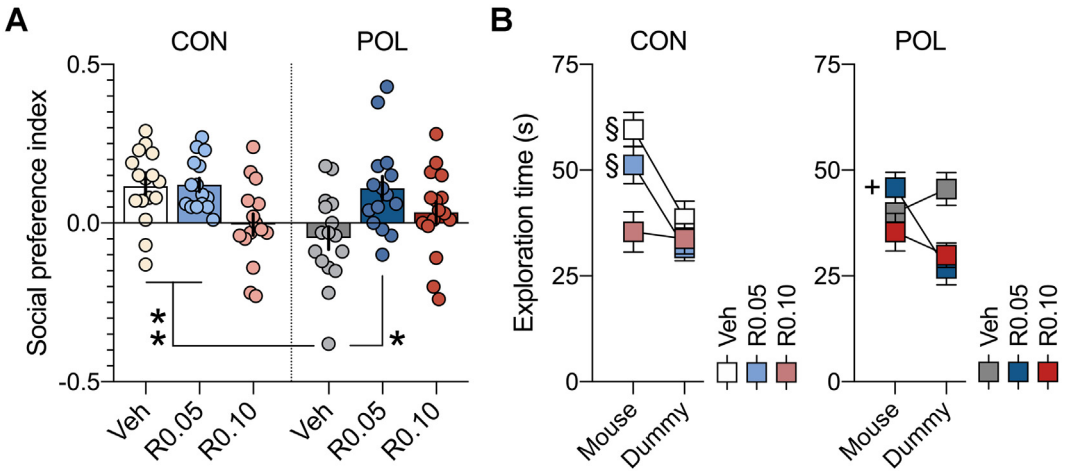

Fig. 3. Effects of chronic risperidone treatment on social deficits in the maternal immune activation model. Poly(I:C)-exposed (POL) and control (CON) offspring were subjected to chronic treatment with risperidone at a dose of 0.05 mg/kg/day (R0.05) or 0.10 mg/kg/day (R0.10), or to corresponding vehicle (Veh) treatment, prior to the social interaction test. (A) Social preference index: Values $>$ 0 represent a preference towards an unfamiliar mouse, whereas values $<$ 0 represent a preference towards an inanimate dummy object. $^{*}p < 0.05$ and $^{**}p < 0.01$, based on post-hoc tests following ANOVA. N = 14–16 per group. Dots represent individual animals, and error bars represent S.E.M. (B) Absolute exploration times of unfamiliar mouse and inanimate dummy object. $^{\S\S}p < 0.01$, reflecting the significant difference between mouse and dummy exploration times in Veh- and R0.05-treated CON offspring; $^{+}p < 0.05$, reflecting the significant difference between mouse and dummy exploration times in R0.05-treated POL offspring. N = 14–16 per group; values are means ± S.E.M.

phase of the AMPH sensitivity test (Fig. 5A). As expected, systemic AMPH administration led to an increase in the animals' locomotor activity, which generally peaked between 20 and 40 min after AMPH treatment (main effect of bins: $F_{(17,1377)} = 68.16$, $p < 0.001$). The AMPH-induced hyperactivity response was significantly potentiated in MIA offspring relative to control offspring (Fig. 5A), as supported by the main effect of prenatal treatment ($F_{(1,81)} = 4.03, p < 0.05$). Both doses of RIS significantly reduced AMPH-induced hyperactivity both in MIA-

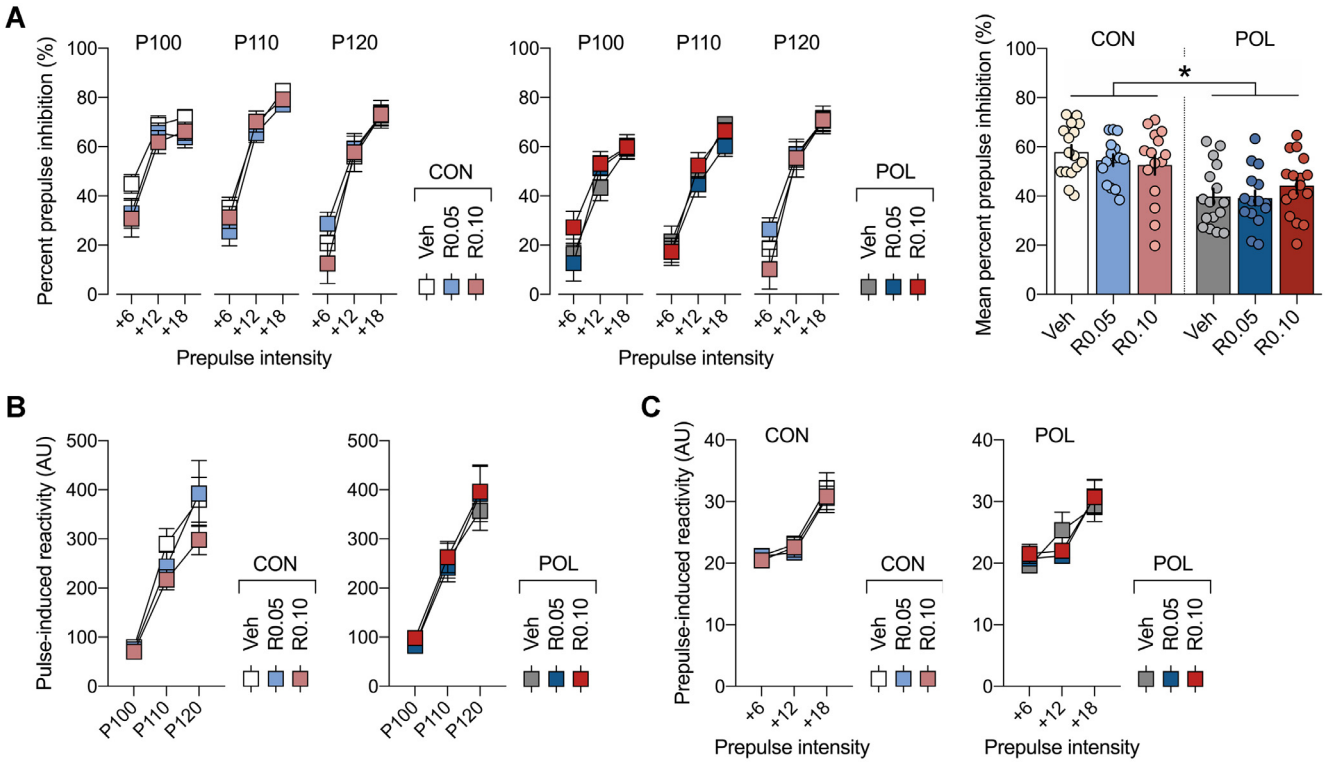

Fig. 4. Effects of chronic risperidone treatment on prepulse inhibition deficits in the maternal immune activation model. Poly(I:C)-exposed (POL) and control (CON) offspring were subjected to chronic treatment with risperidone at a dose of 0.05 mg/kg/day (R0.05) or 0.10 mg/kg/day (R0.10), or to corresponding vehicle (Veh) treatment, prior to the prepulse inhibition test. (A) The line plots show % prepulse inhibition as a function of prepulse intensity (+6, +12 and + 18 dB_A above background of 65 dB_A) for each of the three pulse conditions (P-100, P-110 and P-120, which correspond to pulse intensities of 100, 110 and 120 dB_A). The bar plots depict the mean % prepulse inhibition across the three prepulse intensities in each pulse condition. $^{*}p < 0.05$, reflecting the significant main effect of prenatal treatment based on ANOVA. (B) Pulse-induced reactivity (in arbitrary units, AU) as a function of pulse intensity (P-100, P-110 and P-120, which correspond to pulse intensities of 100, 110 and 120 dB_A). (C) Prepulse-induced reactivity (in arbitrary units, AU) as a function of prepulse intensity (+6, +12 and + 18 dB_A above background of 65 dB_A). For all data, N = 14–16 per group. Dots in bar plots represent individual animals; all line plots represent means ± S.E.M.

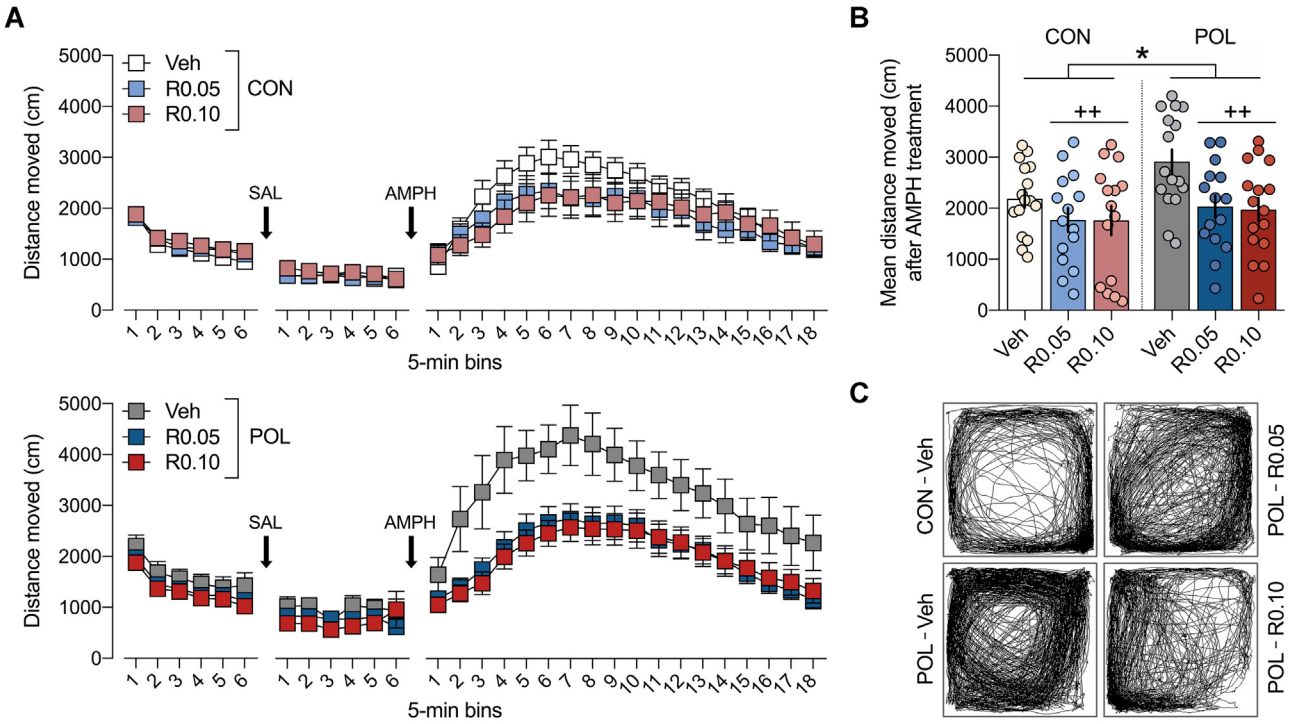

Fig. 5. Effects of chronic risperidone treatment on amphetamine hypersensitivity in the maternal immune activation model. Poly(I:C)-exposed (POL) and control (CON) offspring were subjected to chronic treatment with risperidone at a dose of 0.05 mg/kg/day (R0.05) or 0.10 mg/kg/day (R0.10), or to corresponding vehicle (Veh) treatment, prior to the amphetamine-induced hyperlocomotion test. (A) The line plots show the distance moved (cm) per 5-minute bins during the initial acclimatization phase and subsequent saline (SAL) and amphetamine (AMPH) treatment phases. (B) Mean distance moved during the AMPH treatment phase. $^{*}p < 0.05$, reflecting the significant main effect of prenatal treatment based on ANOVA; $^{++}p < 0.01$, reflecting the significant main effect of risperidone treatment based on ANOVA. (C) Computer-generated travel paths of representative Veh-treated CON and POL offspring and POL offspring treated with risperidone at a dose of 0.05 (R0.05) or 0.10 (R0.10) mg/kg/day. The paths illustrate the total distance traveled during the AMPH phase. For all data, N = 14–16 per group. Dots in bar plots represent individual animals; all line plots represent means ± S.E.M.

exposed offspring and in control offspring (Fig. 5A), leading to a significant main effect of drug treatment ($F_{(2,81)} = 5.05$, $p < 0.01$). The interaction between prenatal treatment and drug treatment failed to attain statistical significance ($F_{(2,81)} = 1.63$, p = 0.20), and so did the three-way interaction between prenatal treatment, drug treatment, and bins ($F_{(34,1377)} = 0.24$, p = 0.98). Likewise, the between-subject factor of sex did not significantly interact with prenatal treatment (prenatal treatment × sex: $F_{(1,81)} = 2.08$, $p = 0.15$), drug treatment (drug treatment × sex: $F_{(2,81)} = 0.07$, $p = 0.93$; prenatal treatment × drug treatment × sex: $F_{(2,81)} = 1.06$, $p = 0.35$) and/or bins (prenatal treatment × sex × bins: $F_{(17,1377)} = 1.32$, $p = 0.11$; drug treatment × sex × bins: $F_{(34,1377)} = 0.81$, $p = 0.76$), showing that the effects of MIA and RIS were independent of the offspring's sex.

4. Discussion

Our study validated a newly developed pharmacological administration method for *per os* treatments in mice. The MDA method described herein makes use of a sweetened condensed milk solution as vehicle for pharmacological substances. Condensed milk is highly palatable to rodents and can mask the possibly aversive taste of pharmacological compounds and solubilizing agents, motivating the animals to learn to consume vehicle and/or drug solutions voluntarily. Indeed, because of its palatable nature, mice quickly (< 3 days) learned to voluntarily drink this solution from conventional micropipettes in the presence of the experimenter. Thus, the MDA technique allows administration of substances without the need for a full restraint or invasive techniques, thereby minimizing the stressful impact on the experimental animals. The latter notion is supported by our findings showing that mice undergoing the MDA procedure did not differ from non-treated animals in terms of plasma levels of the stress hormone, CORT. By contrast, mice treated via oral gavage showed a two-fold increase in plasma CORT levels compared to mice undergoing the MDA procedure or non-treated animals. These results are consistent with previous findings showing significant increases in stress responses after substance administration via oral gavage in mice (Gonzales et al., 2014). Notably, the gavage-induced stress response was still present 30 min post-treatment and could potentially affect neurobehavioral readouts through affecting locomotor activity and anxiety-like behavior (Boyle et al., 2006; Tronche et al., 1999). Conversely, since MDA did not lead to a significant increase in CORT, this method may allow for a less confounded examination of drug effects on neurobehavioral readouts.

Importantly, the MDA procedure is also highly suitable for chronic pharmacological treatments requiring repeated *per os* administration of substances. During 6-week (starting from adulthood) or 11-week (starting from weaning) treatment periods, in which mice underwent the MDA procedure daily, we did not observe any drop out of experimental subjects due to possible saturation to the condensed milk solution and/or injuries acquired during treatment, the latter of which is a typical characteristic of chronic treatment by oral gavage (Arantes-Rodrigues et al., 2012; Burkholder et al., 2012; Turner et al., 2011; Vandenberg et al., 2014). Our results also indicate that the MDA procedure is suitable for preventive treatments in young animals. Indeed, in our 11-week exposure experiment, juvenile mice at PND 21 were already very compliant to the method and showed no differences in consuming the condensed milk solution when compared to older animals. This is particularly relevant to the field of neurodevelopmental research, where preventive interventions are of great interest. Moreover, the MDA technique requires no invasive procedures, unlike methods that call for the implantation of release devices, such as minipumps (Gensler et al., 2012; Theeuwes and Yum, 1976), and it

allows the experimenter to easily tailor the daily administration volumes to each mouse. Another advantage of the MDA procedure is that it is very quick, both in terms of the initial learning phase and the drug/vehicle consummation phase. With regards to the latter, we found that animals only took between 1 and 5 s to consume the condensed milk solution (with or without RIS) when offered from the micropipette.

While administering substances via home-cage food or drinking water represents, similarly to the MDA method, a non-invasive way for chronic *per os* treatments, it can lead to plasmatic profiles that are different to those obtained in human oral treatment regimens. In human settings, substances are typically administered at discrete administration windows and, depending on the substance, lead to a concentration peak in the blood at specific time intervals (Kapur et al., 2003; Shaywitz et al., 1982). Moreover, administration of substances in the home cage drinking water or food is associated with several other drawbacks, which in turn are avoidable with the MDA technique. First, compounds that are insoluble in water have to be pre-dissolved in solubilizing agents such as dimethyl sulfoxide (DMSO) and/or acetic acid, which could produce an aversive reaction when given chronically (Colucci et al., 2008; Turner et al., 2011). Second, these procedures require regular (often daily) measurements of water or food intake to readjust the drug concentrations given via home cage food or water. Third, to accurately measure water or food intake, the animals are often single-housed for the duration of the treatment, which represents, *per se*, a strong stressor, especially when considering chronic manipulations (Arakawa, 2018). Taken together, while our findings do not invalidate the usefulness of experimental procedures in which RIS is given via home cage drinking water (Parikh et al., 2004; Richtand et al., 2011; Rosengarten and Quartermain, 2002) or food, we deem the MDA procedure a precise and time-effective alternative to this approach.

Here, we also provided an initial pharmakokinetic validation of the the MDA procedure. Consistent with pharmacokinetic studies in human subjects (Heykants et al., 1994), we found that RIS was rapidly absorbed following *per os* administration via MDA, with peak plasma levels occurring 30 min after administration. Moreover, our pharmacokinetic analyses showed that there were no differences in the plasma levels of RIS when administered via MDA or oral gavage. Hence, while having the advantage of minimizing stress responses in experimental animals, the MDA procedure leads to desired plasmatic drug profiles similar to conventional oral administration techniques.

Our study further assessed the preclinical validity and utility of the MDA method in a poly(I:C)-based mouse model of MIA, which is frequently used as a model of immune-mediated neurodevelopmental disorders in numerous species, including mice (Brown and Derkits, 2010; Brown and Meyer, 2018; Estes and McAllister, 2016; Kentner et al., 2019). Consistent with numerous previous studies in mice (reviewed in (Brown and Derkits, 2010; Brown and Meyer, 2018; Estes and McAllister, 2016; Kentner et al., 2019), we found that prenatal exposure to poly(I:C) impaired social approach behavior, attenuated PPI of the acoustic startle reflex, and potentiated AMPH-induced hyperlocomotion. These effects emerged similarly in male and female offspring, as the inclusion of the between-subjects factor of sex did not reveal any sex-dependent effects of MIA. Chronic RIS treatment via MDA mitigated the MIA-induced abnormalities in sociability and AMPH responsiveness, further supporting the validity of the MDA method in preclinical psychopharmacological research. To the best of our knowledge, our study is the first to show therapeutic effects of RIS treatment on MIA-induced behavioral dysfunctions when the drug is administered in adulthood. A number of previous studies using MIA models investigated the preventive potential of RIS and other antipsychotic drugs when administered during asymptomatic stages, that is, before the onset of full-blown behavioral dysfunctions in adulthood (Meyer et al., 2010; Piontkewitz et al., 2011, Piontkewitz et al., 2009). While these studies provided evidence for preventive effects of RIS (and other antipsychotic drugs) in MIA models, the present data support the effectiveness of chronic RIS to mitigate abnormalities in social interaction and AMPH hypersensitivity.

Contrary to these effects, however, chronic RIS treatment in adulthood was ineffective in normalizing the MIA-induced deficits in PPI of the acoustic startle reflex. Previous work in rats (Romero et al., 2007) and mice (Vuillermot et al., 2010) suggests that MIA-induced PPI deficiency can be mitigated efficiently by pharmacological inhibitors of dopamine receptors, especially those targeting the D2 subtype. Contrary to the agents used in these previous studies, RIS has a more complex pharmacological profile and is characterized by low D2 receptor affinity (Leysen et al., 1994; Schatzberg and Nemeroff, 2009). Specifically, serotonin 5HT2A and dopamine D2 receptor occupancy predominate at lower and higher doses of RIS, respectively, and the difference between the occupancy of 5HT2A and D2 receptors produced by RIS becomes smaller as the dose is increased (Arnt and Skarsfeldt, 1998; Janssen et al., 1988; Schotte et al., 1996). The low doses of RIS implemented in the present study exert a predominantly 5HT2A receptor antagonistic action, with weak dopamine D2 receptor antagonism (Megens et al., 1994), suggesting that this receptor binding profile is sufficient to rescue social behavior abnormalities and amphetamine hypersensitivity induced by MIA. Consequently, higher RIS doses would be needed for predominant D2 receptor occupancy to occur, suggesting furthermore that a normalization of MIA-induced PPI deficits may require treatments with higher RIS doses than those used in the present study (0.05 and 0.1 mg/kg/day). Our initial dose response studies showed, however, that doses of RIS above 0.1 mg/kg led to sedative effects, which in turn could have confounded the interpretation of some of our behavioral readouts. Therefore, the present study focused on RIS doses that were lower or equal than 0.1 mg/kg/day. The choice of the present RIS dosing regimen was further based on previous preclinical studies assessing the preventive effects of RIS in the MIA and NVHL models (Piontkewitz et al., 2012, 2011; Richtand et al., 2006), which revealed a prevention of social interaction deficits and AMPH hypersensitivity at low doses of RIS comparable to those used here. Future studies are required to explore whether RIS at higher doses is effective in mitigating MIA-induced PPI deficits or not, and if chronic exposure to the drug could somehow lead to a tolerance to its sedative effects, allowing for higher doses to be administered in settings where the behavioral testing is carried out at peak plasma levels of the drug.

We further acknowledge that our study is somewhat limited when considering the portfolio of behavioral tests we chose to include. For example, our study did not investigate the possible impact of chronic exposure to the condensed milk solution on tests that are based on taste and food consumption, as for example the sucrose preference test or the novelty suppressed feeding test. However, given that even chronic administration of condensed milk solution did not affect the weight gain of the exposed animals, we deem it unlikely that the MDA procedure affects their general food intake and thus their motivation to eat or drink rewarding substances in a testing context. Moreover, we did not assess the effects of RIS on other important behavioral abnormalities that characterize the MIA model, such as cognitive deficits alterations in learned fear and affective behaviors. A comprehensive behavioral screening would go beyond the main scope of our study, which was to perform a proof-of-concept validation regarding the validity of the MDA procedure as applied to chronic drug treatments in an established model of neurodevelopmental disorders, which is arguably very sensitive to invasive chronic manipulations. It is also important to emphasize that the observed similarities between the pharmacokinetics profiles of RIS when given in condensed milk via MDA or when administered using other solvents and oral application methods may not necessarily be generalizable to other pharmaceuticals, as they might interact differently with the condensed milk. Furthermore, the condensed milk solution might affect the solubility of certain compounds, rendering the use of such vehicle problematic. While this can be avoided by pre-dissolving the compound in the most appropriate vehicle and then mixing it with condensed milk, an extension to other compounds and model systems would further corroborate the suitability and validity of the

MDA procedure in preclinical pharmacological research. Given the palatable nature of the method, and the higher predisposition of rats to interact with the experimenter and learn non-aversive tasks, we also deem the MDA technique a suitable drug treatment method in preclinical rat models.

In conclusion, our study presents a novel pharmacological treatment method that can be used efficiently for *per os* treatments in small laboratory rodents such as mice. The MDA procedure is not only non-invasive and less stressful for the animals than conventional oral gavages, but also it is easy to implement and cost-effective. This method appears particularly suitable when oral treatments are to be given chronically, as it is without risks of introducing gavage-induced injuries to the digestive tract or infections at the sites of repeated i.p. or s.c. injections. Because the MDA procedure allows the experimenter to adjust the administration volume according to the animal's body weight, it can be efficiently applied to settings where body weight changes occur as a function of age and/or experimental manipulations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.04.015.

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